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Bacterial dispersers along preferential flow paths of a clay till depth profile

Authors: U. S. Krüger[1,2], A. Dechesne[3], F. Bak[1,2], N. Badawi[1], O. Nybroe[2], J. Aamand[1]

[1]Geological Survey of Denmark and Greenland, Copenhagen, Denmark

[2]University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen, Denmark

[3]Technical University of Denmark, Department of Environmental Engineering, Lyngby, Denmark

Corresponding authors: Urse Scheel Krüger: usk@geus.dk and Jens Aamand: jeaa@geus.dk

ABSTRACT

This study assessed the dispersal of five bacterial communities from contrasting compartments along a fractured clay till depth profile comprising plow layer soil, preferential flow paths (biopores and the tectonic fractures below) and matrix sediments, down to 350 cm below the surface. A recently developed expansion of the porous surface model (PSM) was used to capture bacterial communities dispersing under controlled hydration conditions on a soil-like surface. All five communities contained bacteria capable of active dispersal under relatively low hydration conditions (-3.1 kPa). Further testing of the plow layer community revealed active dispersal even at matric potentials of -6.3 to -8.4 kPa, previously thought to be too dry for dispersal on the PSM. Using 16S rRNA gene amplicon sequencing, the dispersing communities were found to be less diverse than their corresponding total communities. The dominant dispersers in most compartments belonged to the genus *Pseudomonas* and, in the plow layer soil, to *Rahnella* too. An exception to this was the dispersing community in the matrix at 350 cm below the surface, which was dominated by *Pantoea.* Hydrologically connected compartments shared proportionally more dispersing than non-dispersing amplicon sequence variants (ASVs), suggesting that active dispersal is important for colonizing these compartments. These results highlight the importance of including soil profile heterogeneity when assessing the role of active dispersal, and contribute to discerning the importance of active dispersal in the soil environment.

IMPORTANCE

The ability to disperse is considered essential for soil bacteria colonization and survival, yet very little is known about the dispersal ability of communities from different, heterogeneous soil compartments. An important factor for dispersal is the thickness and connectivity of the liquid film between soil particles. The present results from a fractured clay till depth profile suggest that dispersal ability is common in various soil compartments and that most are dominated by a few dispersing taxa. Importantly, an increase in shared dispersers among the preferential flow paths of the clay till suggests that active dispersal plays a role in the successful colonization of these habitats.

KEYWORDS

Community motility, liquid film, preferential flow paths, soil, succession

Introduction

Bacterial dispersal in soil has long been considered an important topic of study for microbiologists in various contexts such as bioremediation, ecology, plant protection and community dynamics (1–5). While these studies provide essential insights, they are mostly based on observations from pure culture studies, leaving much still unknown about dispersal in natural soil communities.

Bacteria disperse either passively, *e.g.* by random movement (Brownian motion), transport on plant roots or with water flow, or actively, which requires energy, often using dedicated cellular appendages such as flagella (2, 6–8). In recent studies, there is also an increasing awareness of the potential for cooperative dispersal strategies such as cargo transport of nonmotile bacteria by motile bacterial swarms (3, 9) or interkingdom cooperation with dispersal facilitated by fungi or amoeba (10–13). However, methods for assessing dispersal ability of complex bacterial communities under conditions relevant to soil have only lately become available (14, 15).

Bacteria are aquatic organisms by nature and require an aquatic environment for their life functions (16). In soil, water is also crucial to dispersal because bacterial cells generally need to be fully immersed in liquid to move (2, 17). As a consequence, bacterial dispersal in soil is limited to microhabitats that are interconnected by water pathways, such as the liquid films between soil particles (2, 7). This makes soil water a key factor in bacterial dispersal and consequently in bacterial survival and community diversity. Indeed, connectivity, or more accurately the lack of it, is important for maintaining the huge microbial diversity found in the heterogeneous soil environment (2, 18–21). Connectivity in soil can be considered at different scales, from a microscale at which a single bacterium operates to a macroscale, *e.g.* an agricultural field.

84 At the macroscale, the flow of water in well-structured soils is mainly restricted to preferential
85 flow paths, closely connecting some parts of the soil profile while leaving others isolated (7, 22,
86 23). A “text-book” example of connectivity at the macroscale is agricultural clay tills, where most
87 of the water, primarily from rainfall, moves from the plow layer through preferential flow paths
88 towards groundwater reservoirs. These preferential flow paths comprise a complex system of
89 biopores (mainly earthworm burrows and plant root channels) that are connected to tectonic
90 fractures in deeper layers (22, 24, 25).

91 In clay till, preferential flow paths are fairly well characterized from a geological perspective (24–
92 27), particularly as a result of their potential importance in the leaching of pesticides and other
93 contaminants to groundwater (28). However, from a microbial perspective, much is still unclear.
94 Soils separated by a few meters may have very different community structures (2, 6). Indeed,
95 communities separated by as little as few millimeters can vary in composition, activity, and
96 function, *e.g.* the potential for degradation of pesticides (6, 20, 29). This spatial influence on
97 bacterial communities may be pronounced in clay tills, where the soil profile can be viewed as
98 consisting of spatially isolated compartments, and fracture surfaces and matrix sediment for
99 example, which provide bacterial habitats with vastly different physical and chemical compositions
100 (24–27, 30). These varying conditions can select for different bacteria, leading to differences in
101 community composition (31). Dispersal has the potential to redistribute bacteria and spatially
102 homogenize community composition. While preferential flow paths can be a major route for the
103 passive transport of bacteria through soil (7, 16, 32, 33), the contribution of active dispersal to
104 community assembly processes in soil and sediments has not been explored.

105

One of the most important factors potentially limiting active dispersal in soil and deeper sediments is fluctuating matric potentials and the subsequent loss of connectivity at the microscale, as has been shown in pure culture studies that have highlighted the negative effect of increasingly thin liquid films on flagella-mediated dispersal (18, 34, 35). However, these limitations might not apply to the same extent to the dispersal of environmental communities. Using the novel and expanded PSM method to study bacterial dispersal under controlled hydration conditions on a soil-like surface, Krüger *et al.* (2018) found that part of environmental communities were able to disperse even under conditions previously thought too dry for dispersal (14, 18, 35). According to their observations, rapid dispersal was possible even at a matric potential of -4.2 kPa, but the community response to increasingly negative matric potentials, and thus decreased liquid film thickness and connectivity, have not been investigated beyond that point.

In the present study, the aim was to assess the dispersal potential of bacterial communities from five compartments of a well-defined agricultural soil profile covering the plow layer, deeper preferential flow paths (biopores and tectonic fractures) and adjacent matrix sediments. It was hypothesized that: 1) a sub-community of efficient dispersers is present in each compartment, and 2) these bacteria are frequently shared between hydraulically connected compartments. Furthermore, the effect of low matric potential, and thus low liquid film thickness, on dispersal of a plow layer soil bacterial community was studied and it was hypothesized that: 3) only a fraction of the motile community is able to disperse at low hydration conditions.

Results

Bacterial communities recovered from the soil profile

This study assessed the dispersal of five bacterial communities extracted from five different compartments of a well-defined clay till depth profile (Fig. 1). A newly developed method, the extended porous surface model (PSM), was used in which agar plate imprints are used to reveal the spatial spreading of bacterial communities on a rough hydrated surface resembling soil. This method allows for the recovery and characterization of both the dispersing bacteria and the total community, recovered respectively by pressing a hollowed-out agar plate or a 'full' agar plate onto the PSM surface. The total bacterial communities from the five soil and sediment compartments clearly separated into five clusters on the non-metric multidimensional scaling (NMDS) plot of the community composition from 16S rRNA amplicon sequence data. This was confirmed by PERMANOVA analysis on Bray-Curtis dissimilarities, where 56 % of the variance could be explained by soil compartment ($p < 0.001$) (Fig. 2). Heatmaps and Venn diagrams of the amplicon sequence variants (ASVs) (36) of the total communities also illustrate the different community compositions (Fig. S1- S6). Comparisons between the original soil community, the inoculum (Nycodenz extractions) and the cultivable communities on the full plates and reference plates (inoculum placed directly on agar plate) confirm the expected cultivation bias (Fig. S7 and S8). Yet, in general, the total cultivable communities retained a high level of diversity, with representatives of 109 unique genera (belonging to 5 different phyla), across all compartments plus 161 ASVs that were not identifiable at the genus level (Table S1). 27.7 % and 8.7 % of the genera in abundance >0.1 % in the biopores and plow layer soil communities, respectively, were recovered on the full plates pressed onto the PSM incubated for 48h at -3.1 kPa. Similar values were observed for the -0.5 kPa

24 h samples (Table S2). Additionally, the ASVs found on the full plates represented 10 % and 1 % of the original community from the biopores and plow layer soil (Table S3). This signifies that the applied method was able to recover a substantial part of the diversity present in the original soil communities.

The genera *Pseudomonas*, *Flavobacterium* and *Pedobacter* dominated the total communities of the plow layer, biopores, and matrix at 80-120 cmbs (cm below surface) (Fig. S1- S3), while in the fracture community at 300-350 cmbs, *Flavobacterium* was replaced by *Arthrobacter* (Fig. S4). The community from the deep matrix sediment at 300-350 cmbs was dominated by the genus *Pantoea*, followed by *Pseudomonas*, *Chryseobacterium* and *Stenotrophomonas* (Fig. S5). The moisture conditions on the PSM model (-0.5 and -3.1 kPa) had only a minor influence on the total bacterial community composition, contributing just 7 % of the variation in the PERMANOVA analysis of Bray-Curtis dissimilarities ($p<0.001$) (Fig. 2 and Fig. S1-5). In conclusion, the soil communities recovered from the PSM were distinctly different, although they shared some dominant genera.

Community dispersal potential and identity of major dispersers

Rapid dispersal of bacteria was observed for all soil and sediment communities in wet conditions (-0.5 kPa). Except for the plow layer community, there was a clear tendency towards slower dispersal and lower surface coverage scores in dry conditions (-3.1 kPa) compared to wet conditions, indicating dispersal limitation in dry conditions (Fig. 3).

Using 16S rRNA gene amplicon sequencing, the dispersing bacteria from the extracted soil and sediment communities were identified. The composition of these dispersing communities was

172 then compared to the total bacterial communities. Both the Shannon diversity and Faith's
173 phylogenetic diversity indices showed that the total communities were more diverse than the
174 dispersed communities, and that the dispersers had a narrow phylogenetic diversity (Fig. S9 and
175 S10). The Shannon diversity index also revealed a lower diversity in dry (-3.1 kPa) compared to wet
176 conditions for all dispersed communities, except for the matrix sediment at 80-120 cmbs, where a
177 high variation between replicates was seen. Dispersing bacteria predominantly belonged to the
178 genus *Pseudomonas* in all but one community at -0.5 kPa. Additionally, under these wet
179 conditions, the plow layer and the biopore dispersers shared a high relative abundance of
180 *Rahnella*, *Paenibacillus*, *Lysinibacillus* and *Kluyvera* (Fig. 3). In dry conditions, *Pseudomonas* almost
181 completely dominated the dispersed communities, except for the matrix soil at 300-350 cmbs.
182 Here *Pantoea* was dominant at -0.5 kPa, while at -3.1 kPa *Pantoea* and *Pseudomonas* were
183 represented equally. In general, the dominant disperser genera were also represented in the total
184 community, but they were greatly enriched in the disperser communities.

185

186 On an NMDS plot (Fig. S11 and S12) the dispersed communities separated from the total
187 communities, as confirmed by PERMANOVA analysis on Bray-Curtis dissimilarities, explaining 9 %
188 and 11 % of the variance for -0.5 and -3.1 kPa (all $p<0.001$) respectively. However, the strongest
189 effect was still attributed to the compartment type, explaining 47 % and 28 % of the variance in
190 wet and dry conditions. Additionally, there was a significant, but moderate, interaction between
191 dispersed/total community and soil compartment (14 %, $P< 0.001$, for -0.5 kPa, 24 h and 15 %,
192 $P<0.001$, for -3.1 kPa, 48 h respectively). Significant differences in homogeneity between the
193 dispersed and total communities was found using Betadisperser followed by ANOVA, which tested

194 whether the dispersion of a group from its median was different from the dispersion of other
195 groups (F=5.9 , P< 0.05, for -0.5 kPa 24 h and F= 7.1, P< 0.01 for -3.1 kPa 48 h). Hence, the
196 dispersed communities had a significantly greater variation than the total communities, indicating
197 a stochastic element in the identity of the bacterial dispersers.

198

199 **Connectivity of dispersing communities from preferential flow paths and matrix**

200 A closer look at the ASVs in the dispersed communities (Fig. 4 A, B and Tables S4-S5) revealed
201 many ASVs shared between the plow layer, biopores and fracture communities. The number of
202 shared ASVs was generally much higher in wet conditions than in dry conditions. The most
203 common genus amongst the shared dispersers between the three communities of the plow layer,
204 biopores and fractures under both hydration conditions was *Pseudomonas* (10 shared ASVs at -0.5
205 kPa and -3.1 kPa), but *Buttiauxella* was also represented (1 shared ASV at -0.5 kPa and -3.1 kPa).
206 The one ASV shared between all compartments under wet conditions belonged to the genus
207 *Pseudomonas*.

208

209 Comparing the percentages of shared ASVs between dispersing and non-dispersing bacteria from
210 the preferential flow paths also revealed a very clear picture of the dispersers being more shared
211 than non-dispersers (Table 1). It should be noted that what are referred to as “non-dispersers” are
212 actually the ASVs in the total communities minus the ASVs observed among the dispersers. This
213 group may therefore also contain some slow dispersers, which were not quick enough to be
214 detected among the dispersers. The proportion of shared dispersers was significantly higher than
215 the proportion of shared non-dispersers in the preferential flow paths for both wet and dry

conditions, although in dry conditions this was only significant for the shared communities between the plow layer and fractures. In contrast to the greater sharing of dispersers along the preferential flow paths, there was no significant preferential sharing of dispersers between the preferential flow paths and the adjacent matrix sediments. Indeed, in some cases there was even greater sharing of non-dispersing ASVs between these compartments, as was also the case for vertical sharing between the matrix 80-120 cmbs and matrix 300-350 cmbs sediment communities.

Response to increasingly negative matric potentials

To test the effect of increasingly negative matric potentials on the dispersal ability of a soil community, the community extracted from the plow layer soil was exposed to matric potentials from -0.5 to -8.4 kPa. Interestingly, dispersal was seen even in the driest conditions, but the Shannon diversity index of the dispersing community decreased as the conditions became dryer (Fig. 5), with only very few genera present in dry conditions (Fig. 6 and Fig. S13). Furthermore, Faith's diversity index extended the previous results, showing that the narrow phylogenetic distribution of the dispersed communities became narrower in even dryer conditions (Fig. S14).

In general, dispersal was increasingly restricted at matrix potentials of -4.1 kPa and below (Fig. 6), with no replicates dispersing beyond the 15-20 mm section for matric potentials of -6.1 and -8.4 kPa. An element of randomness seemed to be involved in the identity of the dispersers at -4.1 to -8.4 kPa, with *Pseudomonas* still being prominent, but in some replicates *Cupriavidus, Bacillus*, or *Pseudoduganella* were also dominant dispersers.

237 The element of randomness at decreased matric potentials was also supported by visual
238 observations of the colonization patterns on the surface of the agar plates (Fig. S15). While at -0.5
239 and -3.1 kPa the patterns were characterized by a relatively uniform spread of bacteria from the
240 inoculation point to the edge of the plate at -6.3 and -8.4 kPa, dispersal was limited to a few
241 corridors.

242

243 ## Discussion

244 **Dispersal potential and disperser identity in communities from fractured clay till**

245 While the role of passive transport is well established (2, 32, 33), the importance of active
246 dispersal in soil has been debated for many years (2, 3, 7, 17). Until recently there was no
247 experimental platform to screen for active dispersal at the community level (14, 15). The present
248 study assessed the dispersal of five bacterial communities from matrix sediments and preferential
249 flow paths of a clayey till.

250 The soil and sediment compartments studied here harbored different bacterial communities,
251 reflecting the very heterogeneous nature of clay till profiles and confirming the existence of
252 distinct compartments (30, 31). Dispersing bacteria were found in the plow layer and in all deeper
253 sediments, pointing to the importance of active dispersal in these environments, though these
254 dispersers were not dominant in the total communities (14, 15). *Pseudomonas* was the dominant
255 disperser in the top soil, in agreement with results from the few comparable studies available (14,
256 15). Members of the *Pseudomonadacea*e family have also been found to be early colonizers of
257 plant litter in an agricultural field, also suggesting that *Pseudomonas* is a key disperser in the soil

environment (37). In the literature, pseudomonads are known to be efficient dispersers, employing various adaptations such as swimming, swarming and sliding motility (35, 38–40). Additionally, it has been suggested that the ability of *Pseudomonas* to disperse even in dry conditions could be linked to their ability to produce biosurfactants, which can facilitate surface dispersal, especially in fluctuating hydration conditions (14, 41–43). However, the production of surfactants might be linked to specific habitats such as the rhizosphere (41, 44), and the presence of surfactants would therefore have to be proven in soil-like conditions.

In general, the dispersing bacterial communities of the soil and sediment compartments had a narrow phylogenetic distribution and many dispersing taxa were shared between the compartments. These dispersers had several genera in common with dispersers from other plow layer soils (14, 15). Besides *Pseudomonas*, these were *Paenibacillus*, *Flavobacterium* and *Janthinobacterium,* as well as *Rahnella* and *Pantoea,* the latter two belonging to the enterobacteria, a group identified as the most abundant disperser in a previous study (15). While *Pseudomonas* flagellar swimming dispersal and *Flavobacterium* gliding dispersal on surfaces have been studied extensively, mainly in pure cultures (18, 35, 45–48), there needs to be a greater focus on the mode of dispersal of other genera, *e.g. Pantoea,* which was found to be dominant in the deep matrix sediment at 300-350 cmbs in the present study.

Dispersal rates were severely inhibited in conditions dryer than -3.1 kPa, because smaller liquid film thickness on the ceramic surface prevent active dispersal, as previously demonstrated for both pure bacterial cultures (18, 35) and soil and lake microbial communities (14).

Bacterial dispersers in preferential flow paths *versus* matrix sediments

The high percentage of shared dispersers between the communities derived from the preferential flow path compartments compared to the matrix sediments indicated that the interchange of dispersing genera is more common along hydrologically connected compartments. This concept is illustrated in Figure 7. As the preferential flow paths are enriched with dissolved nutrients, oxygen and organic carbon transported from the surface by the flow of water (27, 49, 50), they can provide an attractive habitat for soil bacteria. While it has previously been shown that preferential flow paths have the potential to be a major route for the passive transport of bacteria (7, 16, 32, 33), the current results support the notion that part of the bacterial communities in the flow paths can also take advantage of active dispersal to spread through and colonize these habitats. This was especially interesting as the benefit of active motility in the presence of water flow was not obvious. Indeed, it might have been expected that the benefit of active motility would be more prominent in the matrix soil where flow is absent.

The limited number of shared dispersers between the flow paths and the adjacent matrix sediments, especially at 300-350 cmbs (Table 1), may be due to limited connectivity. Given the small particle size of clay particles (< 2µm), the pore space in dense clay sediment is generally very small (7), probably impeding bacterial dispersal. Indeed the porosity of the clay till at the current study site decreases with depth (51). Although some bacterial pure cultures are known to be able to swim through apertures as small as 1.1 µm (52), the small pore size and low connectivity of matrix clay tills likely form a barrier to the exchange of bacteria, in particular at 300-350 cmbs. For comparison, deep fractures are reported to have apertures of 100 µm (22) and biopores may have

diameters of 8-10 mm, leaving ample space for bacterial dispersal subject to the presence of sufficient liquid films. Furthermore, fractures may be coated with metal oxide precipitates such as iron oxides, which can be almost impermeable to water (27, 53) and therefore probably also a hindrance for bacterial dispersal.

It is tempting to speculate that bacterial communities of dense clay matrixes are islands that have little contact with nearby communities. Water percolation through clay till matrix sediments is very limited (22, 23), thereby providing little input of nutrients and organic carbon to the bacteria. The low amount of nutrients in deep sediments (54) may also inhibit active dispersal as nutrient limitation can greatly decrease the fraction of motile bacteria (55). We recognize that the method applied in this study is limited to the fraction of bacteria able to grow under the selected growth conditions excluding contribution of microbes that cannot be active under the conditions of our assay (e.g. strict anaerobes). Nonetheless, while the current study was limited to exploring subgroups of the total diversity of soil bacteria present along the preferential flow paths of a clay till depth profile we believe that we are uncovering important processes relating dispersal potential and connectivity in the heterogeneous soil environment. The observed patterns of intensified cell exchange affected by dispersal potential and soil compartment should apply to other bacteria as well as the principles of soil physics will apply irrespective of bacterial taxonomy.

Dispersal at low matric potentials

In unsaturated soil, low matric potentials are known to negatively affect bacterial dispersal (2, 18, 35). Here, the matric potential on the PSM was extended to -8.4 kPa, the lowest possible without using a pressurized version of the PSM (56), in order to investigate how low hydration conditions

affect active dispersal of soil bacterial communities. The finding of active dispersal even at -8.4 kPa, albeit at a decreased rate, was surprising because recent measurements of liquid films on the PSM have shown rapid thinning and disconnection of the liquid films at matric potentials exceeding -2.0 kPa (18), causing severe inhibition of dispersal, as demonstrated for bacterial pure cultures (18, 35, 46). However, due to residual surface roughness on the PSM it is still possible to observe rare thicker liquid films (≥ 5 µm) at -2.0 kPa (18). Visual analysis of the agar plates suggested that dispersal at the lowest matrix potentials of -6.3 to -8.4 kPa occurred along a few of such narrow liquid film corridors on the rough surface. At a decreased matric potential (-4.1 to -8.4 kPa), *Bacillus* and *Pseudoduganella* rather than *Pseudomonas* were major dispersers in some replicates, indicating a stochastic element with regards to which bacteria disperse when water film thickness becomes limited. Due to the complex heterogeneous nature of soil we speculate that there could also be some open dispersal corridors available in natural soil even under relatively dry conditions. One known option is the use of the thin liquid films surrounding fungal hyphae (i.e. fungal highways) (57, 58). It has been suggested that the abundance of mycelial networks in soil is part of the explanation for the maintenance of the otherwise costly flagella in soil bacteria (11, 59).

It has been claimed that active motility is limited in soil mainly due to dry and unsaturated conditions, which confines active dispersal to transient wet periods, *e.g.* during rain events (2, 7, 21). These claims have been supported in part by experimentation done using the porous surface model, showing that bacterial flagellar motility is restricted to a narrow range of high matric water potentials (18, 35). While the relationship between matric potential on and liquid film thickness on

the PSM differs from that in soil, it is relevant to ascertain if the range of matric potentials found in soil is compatible with flagella powered swimming. According to data from the Danish Pesticide Leaching Assessment program (PLAP) (60, 61) in fractured clay till, which is a common soil type in Denmark (26), the matric potential of Danish agricultural top soils can fluctuate between -5 and -1500 kPa while deeper clayey matrix sediments (from 60 cmbs and down) remain water-saturated (~ 0 kPa) most of the time (61–63). There should therefore be sufficient liquid films in subsurface clay till to allow active dispersal unless low pore connectivity and fracture coatings create physical barriers that cannot be overcome.

Conclusions

This study demonstrated that different compartments of a heterogeneous clay till depth profile harbor bacterial communities that are capable of dispersing in low hydration conditions. The dispersers show narrow phylogenetic diversity and are dominated by pseudomonads and enterobacteria. Active dispersal occured even within thin and poorly-connected liquid films on the surface of the PSM at matric potentials of -6.3 to -8.4 kPa. These results indicate that active dispersal ability is widespread in soil and sediment communities. An increased proportion of disperser ASVs shared between highly connected compartments (*e.g.* preferential flow paths) points to a role for active dispersal in the spread through, and colonization of, these habitats. Fewer shared disperser ASVs between the preferential flow paths and the matrix sediments illustrated that low porosity of clay tills and metal oxide-coated fracture walls might be barriers to the exchange of bacteria, leaving matrix bacterial communities relatively isolated.

Materials and methods

Soil sampling

Soils were sampled over a three-day period in September 2016 from an agricultural field (Anthric Luvisol) in Lund, Denmark (55°14'49''N, 12°17'24''E) (51). The adjacent field has recently been included in the Danish Pesticide Risk Assessment Program (PLAP)(51, 64). The soil is characterized by clay till and boulder clay, with a very pronounced fracture system down to at least 6 m depth. While the biopores, dominating the top 150 centimeters below the surface (cmbs), mainly consist of earthworm burrows and decayed root channels, the fractures below are mainly of tectonic origin.

A multi-bench excavation down to 6 m depth allowed the sampling of sediment from different depths. Soil was sampled from the plow layer (0-20 cmbs), biopores (80-120 cmbs), matrix sediment next to the biopores (80-120 cmbs), oxidized iron-rich red fractures (300-350 cmbs) and matrix sediment next to these fractures (300-350 cmbs) (Fig. 1). Soils were collected as composite samples, *i.e.* as small subsamples combined into one pooled sample for each of the five soil and sediment compartments. One composite sample equaled *ca*. 15-30 subsamples per soil compartment, except for the biopore samples, which consisted of *ca*. 70 subsamples. The subsamples were combined into one composite sample per compartment to ensure sufficient soil from biopores and fractures for further analysis. Samples were secured by carefully removing the outer layer of the soil profile with a knife to avoid cross contamination. Hereafter the freshly exposed soil and sediment were subsampled (carefully scraped off) with a small spoon and stored at 5 °C.

Extraction of soil bacteria

The soil and sediment samples from each compartment were homogenized by sieving (2 mm), and mass reduction for laboratory subsampling was performed by bed blending, as described in the "Representative Sampling Horizontal Standard" (65) and by Kardanpour *et al.* (2015) (66). This resulted in 25 g composite soil or sediment sample for each experimental setup.

The soil bacterial community from each compartment was extracted using Nycodenz density gradient centrifugation as in (67), except for the final cell density determination, which was performed directly using a Thoma counting chamber. Cell densities of the extracts were adjusted to 0.8×10^6 cells μl^{-1} in 0.9 % NaCl solution. The soil bacterial extracts were kept at 4 °C overnight before inoculation on the ceramic discs of the extended porous surface model system.

Dispersal potential of environmental communities using the extended porous surface model

An extended version of the porous surface model (PSM) (14) was used, where the original PSM model (46) had been further developed to encompass the dispersal of non-fluorescent complex communities extracted from environmental samples. The method allows communities to disperse under controlled hydration conditions from the center of a porous ceramic disc (diameter = 41.3 mm, thickness = 7.1 mm, maximum pore size <1.5 µm, 1 bar bubbling pressure; Soilmoisture Equipment Corp., Santa Barbara, USA), mimicking a rough soil surface. Imposing suction on the ceramic disc allows for precise control of the liquid film thickness on its surface. The liquid medium used in the PSM was 25 % R2B (Alpha Biosciences, Baltimore, MD). Each experimental setup allowed for the parallel incubation of 9-11 PSMs.

411 Each PSM was inoculated with 10 µl of bacterial extract placed as 1 µl drops at the center of the
412 ceramic disc. Although the inocula for the PSM were adjusted to the same cell densities according
413 to Thoma counts, the cultivable fraction was generally lower in deep sediment samples compared
414 to plow layer and biopore soil. CFU numbers were highest in the plow layer (*ca.* 11,250 CFU) and
415 biopores (130,000 CFU), and decreased in the matrix at 80-120 cmbs (1,000 CFU), red fractures
416 (1,125 CFU) and matrix sediment from 300-350 cmbs (750 CFU). Colonies were enumerated on
417 25 % R2A plates (Fluka R2A; Sigma-Aldrich, St. Louis, MO) after incubation at 25 °C for 48-72 hours.
418 All plates were amended with 100 mg l^{-1} Delvocid to inhibit fungal growth (Natamycin, DSM food
419 specialties, Delft, The Netherlands).

420 After inoculation, the discs were brought to matric potentials of -0.5 or -3.1 kPa and incubated at
421 room temperature for 24 or 48 hours before sampling. After incubation, the bacteria were
422 recovered from the surface of the ceramic disc by means of an agar plate lift. This is described in
423 detail in Krüger *et al.* (14) In brief, to visualize the colonization on the ceramic disc, a series of agar
424 plates were used to cover different sections of the ceramic surface. The agar plate series consisted
425 of small flat 25 % R2A plates containing 20 g agar l^{-1} (Star^{TM}Dish diameter, 40 mm; height,
426 12.5 mm; Phoenix Biomedical Products, Mississauga, Canada), with holes in four sizes. Sampling
427 was achieved by starting with the plate with the largest hole size, 25 mm, followed by 20, 15,
428 11.5 mm, and ending with the pressing of a full agar plate (full plate). The extent of colonization of
429 the ceramic disc was quantified by evaluating the coverage of bacterial growth on the individual
430 agar plates, after 72 h incubation at 25 °C, and dividing it into four categories: 1-25, 26-50, 51-75
431 and 76-100 % coverage.

For each series of five pressed plates, the fastest-dispersing bacteria from the environmental communities were then identified, *i.e.* the colonies of the pressed agar plate the furthest from the point of inoculation (the plate with the largest hole size) that presented growth (referred to as the "dispersers" or "dispersing community"), and the total community present on the full agar plates, by 16S rRNA gene amplicon sequencing. The full plate represented the cultivable community developing on an agar plate covering the entire ceramic plate, *i.e.* both dispersing and non-dispersing bacteria. Additionally, for each separate experiment and soil, a no-motility reference plate, shortened to "reference plate", was made by drop-plating 10 µl of each inoculum directly onto the center of a small 25 % R2A plate with 20 g agar l^{-1}, which provided conditions that are not conductive for flagellar motility and are not influenced by the PSM (34). All bacteria were washed off the agar plates using 0.9 % NaCl following the procedure described by Krüger *et al.* (2018) (14). For comparisons with the dispersed communities, total communities present on the full plates were generally preferred, as they captured the double cultivation step (both on the PSM and on the agar plates). However the reference plates were also valuable because they gave an indication of what could be cultivated upon direct inoculation on the agar plates. The cell suspensions from the pressed plates and the reference plates (plate wash) as well as the original Nycodenz extracts were all stored at -80 °C before further processing.

Porous surface model with increasingly negative matric potentials

To achieve matric potentials down to -8.4 kPa, the PSM assembly was slightly modified. To limit the amount of air entering the system, the PSM tubing was tightened and partly replaced with stainless steel. To further limit the formation of air bubbles that can form in the medium at

lowered matrix potentials, the ceramic plates were degassed for 24 h using a vacuum pump, and the 25 % R2B medium was degassed for 20 minutes in an ultrasound bath. PSMs were assembled submerged in degassed medium.

DNA extraction and sequencing

DNA was extracted using the DNeasy Powerlyzer Powersoil kit (Quiagen; Hilden, Germany) following the manufacturer's protocol with a few adjustments, as in Krüger *et al.* (2018). The DNA concentrations were measured on Qubit 2.0 (Life Technologies, Invitrogen; Carlsbad, USA) and samples stored at -80 °C until sequencing. The DNA was PCR-amplified using the primer set 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GACTACHVGGGTATCTAATCC-3') (68) targeting the hypervariable V3-V4 regions of bacterial 16S rRNA genes. The purified PCR products (2 x 300-bp reads) were sequenced on the Illumina Miseq platform by Macrogen (Seoul, South Korea).

The raw 16S rRNA gene amplicon sequences were processed using the DADA2 pipeline (69) with default parameters. Sequence classification was based on the SILVA prokaryotic reference database version 123 (70). A total of 7.2 million sequences passed the filtering steps, representing an average of 60,500 sequences per sample.

Data analysis and statistical methods

Data analysis of sequences and statistics was computed in R (71). The Shannon diversity index was calculated using the "plot_richness" function in the phyloseq package (72). Faith's phylogenetic diversity (PD) was calculated with the "pd.query" function in the PhyloMeasures package (73).

475 Prior to calculating the PD, samples were rarefied to an even depth (mean of 10 iterations) using
476 the "rarefy_even_depth" function in the phyloseq package. Heatmaps were plotted using the
477 "amp_heatmap" function in the ampvis2 package (74). Venn diagrams were plotted using the
478 function "venn" from the gplots package (75). Non-metric multidimensional scaling (NMDS)
479 ordination was undertaken on Bray-Curtis dissimilarities using the "ordinate" function in the
480 phyloseq package. PERMANOVA and analysis of multivariate homogeneity of group dispersions
481 (variances) were computed using the "adonis" and "betadisper" functions in the vegan (2.4-6)
482 package (76), with 999 permutations. Differences in the proportions of shared ASVs between
483 communities were tested using Fisher's exact test in R (71).

484 Additional statistical analysis was undertaken using Sigmaplot 13 (Systat Software, Inc., San Jose,
485 CA).

486 Differences in Shannon diversity indices between total communities and the fastest dispersers was
487 tested using one-tailed, one sample t-tests (testing for subtracted differences greater than zero).
488 The effects of matric potentials were tested using two-tailed t-tests. P values of < 0.05 were
489 considered significant.

490 **Accession number(s).** All sequencing data have been deposited as an NCBI BioProject under
491 accession number PRJNA483533

492

493 ## Acknowledgements

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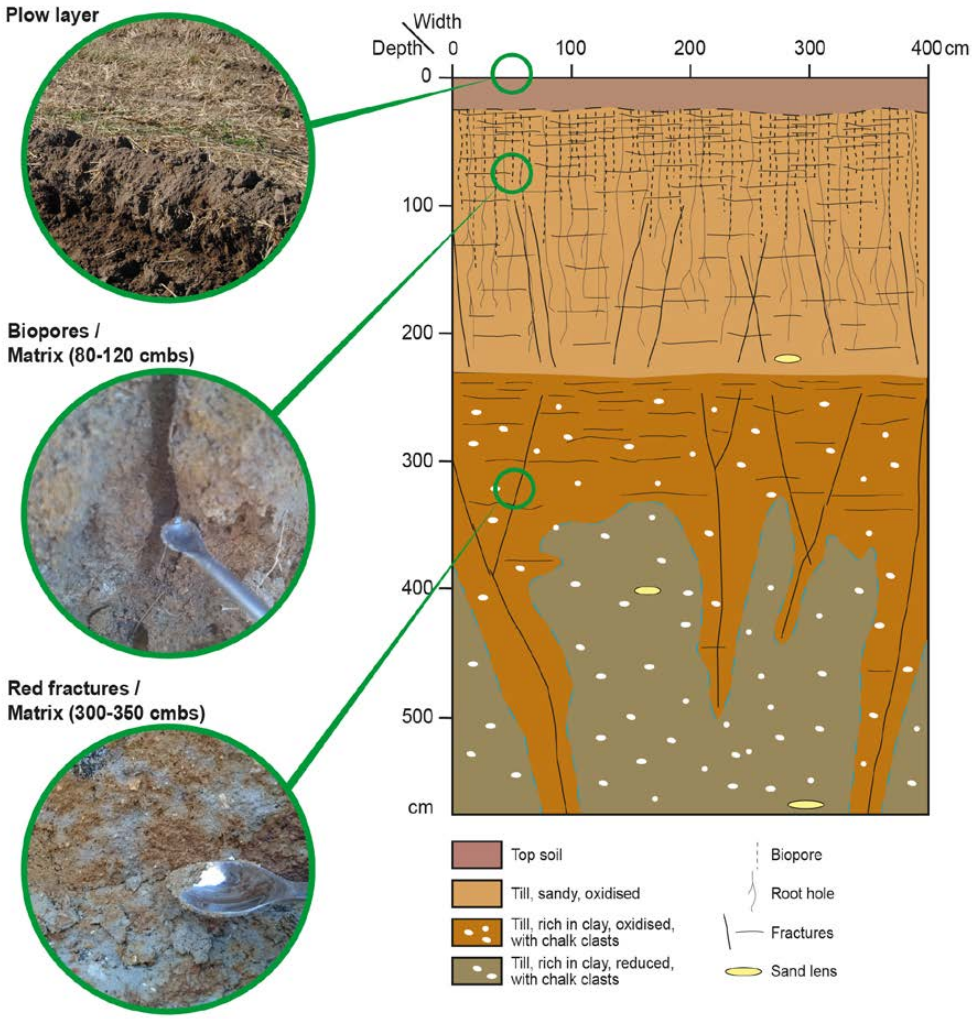
684 **Figures**



685

686 **FIG 1 Schematic illustration of the soil profile with highlighted sampling points.** Plow layer
687 samples were obtained from 20 cmbs (cm below surface). Biopores and matrix sediment samples
688 were from 80-120 cmbs, and red fractures and matrix sediment were sampled from 300-350
689 cmbs. The illustration is adapted with permission from a report from the Danish Pesticide Leaching
690 Risk Assessment Program (PLAP) (64).

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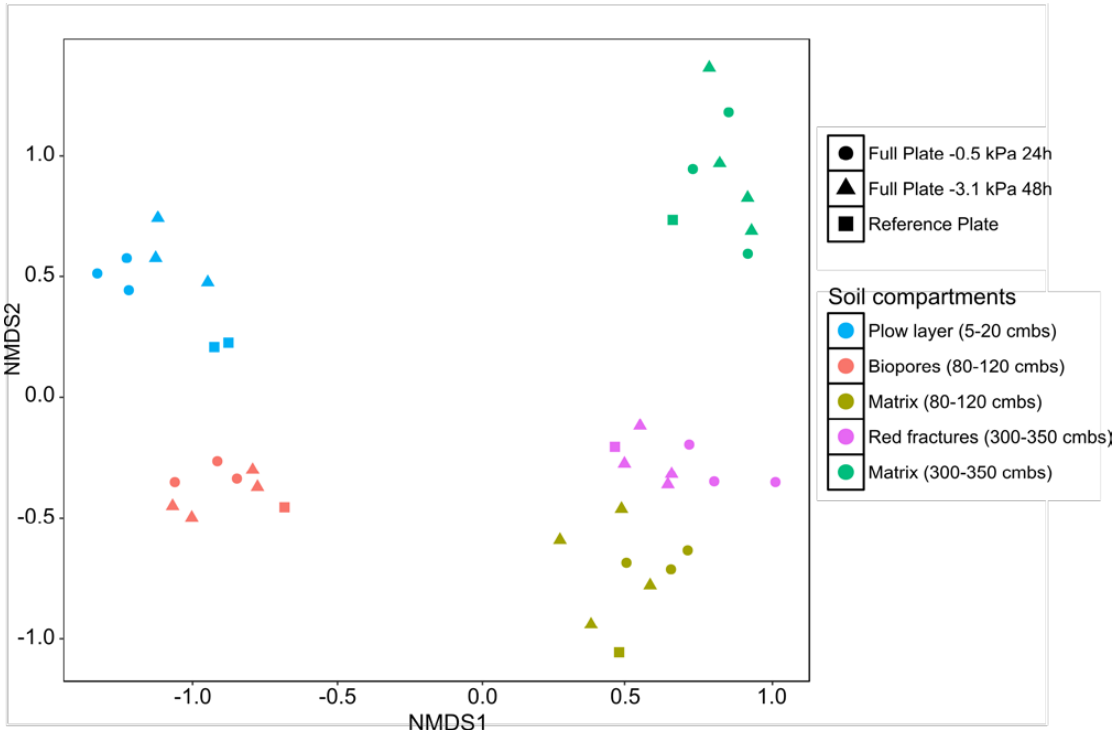
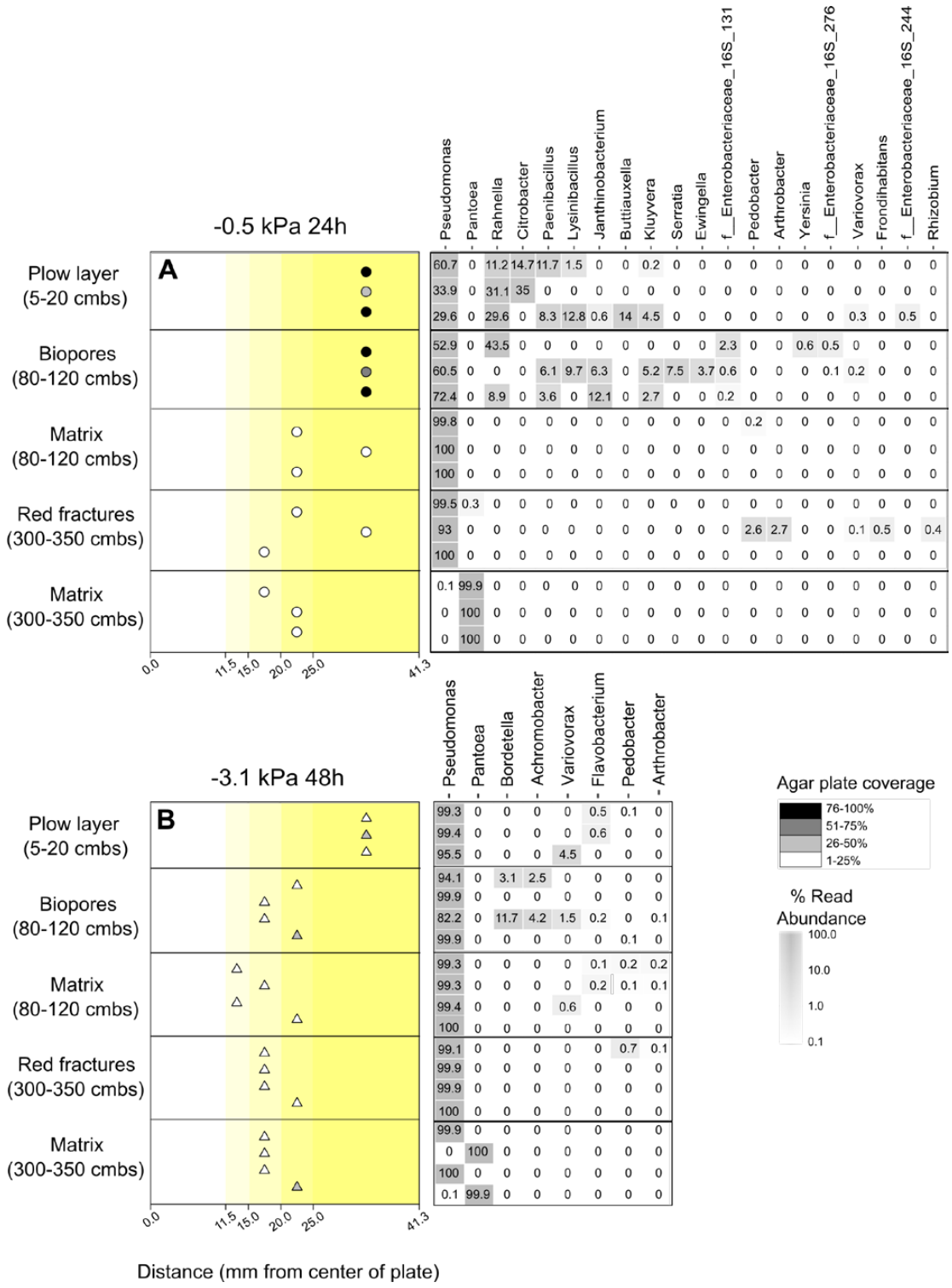


692

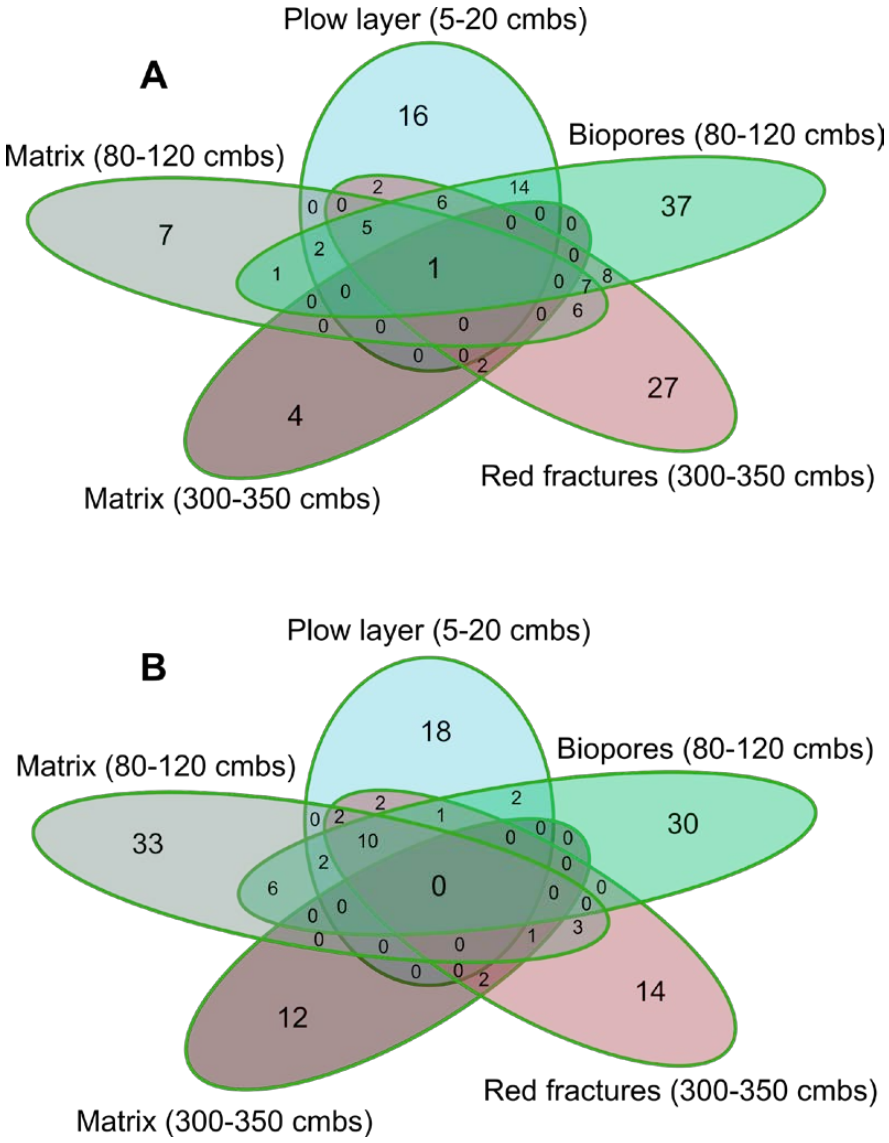
693 **FIG2** NMDS plot of the composition of the total communities derived from five compartments of a
694 well-defined soil profile. Stress = 0.13. Bray-Curtis dissimilarities calculated from 16S rRNA genes.
695 The total communities were tested at two matric potentials in the PSM experiments, -0.5 kPa
696 (circles) and -3.1 kPa (triangles), and recovered on full agar plates (full plate). The motility-

restricted controls (reference plate) are marked with squares. Replicates are depicted as separate dots.



701

FIG 3 Dispersal and composition of communities derived from five compartments of a well-defined soil profile and incubated at matric potential -0.5 kPa for 24 h (**A**) and -3.1 kPa for 48 h (**B**). Left: Symbol shading depicts bacterial coverage of the pressed agar plate, giving an indication of the extent of colonization. The distances shown are ranges, *e.g.* colonies were observed on the agar ring at a distance of between 11.5 and 15 mm from the inoculation point at the center. Right: Heatmap of the relative abundance of the most dominant genera among the dispersing bacteria across five soil communities. The replicates are depicted as separate dots and replication numbers varied from three to four.



712

713

714 **FIG 4** Venn diagrams depicting the shared and unique ASVs between the dispersed communities

715 from five compartments of a well-defined soil profile. A) Communities exposed to

716 -0.5 kPa for 24 h, with a total of 145 unique ASVs. B) Communities exposed to -3.1 kPa for 48 h

717 with a total of 138 unique ASVs.

718

719

Table 1.
Shared dispersing and non-dispersing ASVs between communities derived from five compartments of a well-defined soil profile

		Percentage of shared dispersers[a]	Percentage of shared non-dispersers[b]	*P* value Fisher's exact test
Preferential flow paths				
Plow layer *vs.* biopores	-0.5 kPa	28.9 %	12.2 %	P< 0.001
	-3.1 kPa	20.0 %	15.0 %	0.2892
Biopores *vs.* fractures	-0.5 kPa	22.9 %	5.6 %	P< 0.001
	-3.1 kPa	14.7 %	7.6 %	0.1042
Plow layer *vs.* fractures	-0.5 kPa	14.6 %	3.8 %	P<0.01
	-3.1 kPa	26.3 %	6.9 %	P<0.001
Preferential flow path *vs.* matrix				
Biopores *vs.* matrix at 80-120 cmbs	-0.5 kPa	17.0 %	13.6 %	0.5636
	-3.1 kPa	20.0 %	12.6 %	0.1093
Fractures *vs.* matrix at 300-350 cmbs	-0.5 kPa	4.4 %	13.3 %	0.0826
	-3.1 kPa	6.4 %	17.8 %	0.07794
Matrix *vs.* matrix				
Matrix 80-120 *vs.* matrix 300- 350 cmbs	-0.5 kPa	2.9 %	10.1 %	0.2712
	-3.1 kPa	1.4 %	11.9 %	P< 0.05

720

721 [a] Dispersed bacteria recovered the furthest from the inoculation point (at least 11.5 mm).

722 [b] Non-dispersing bacteria were calculated by subtracting the unique ASVs observed in the

723 dispersed community from the ASVs observed in the total community.

724

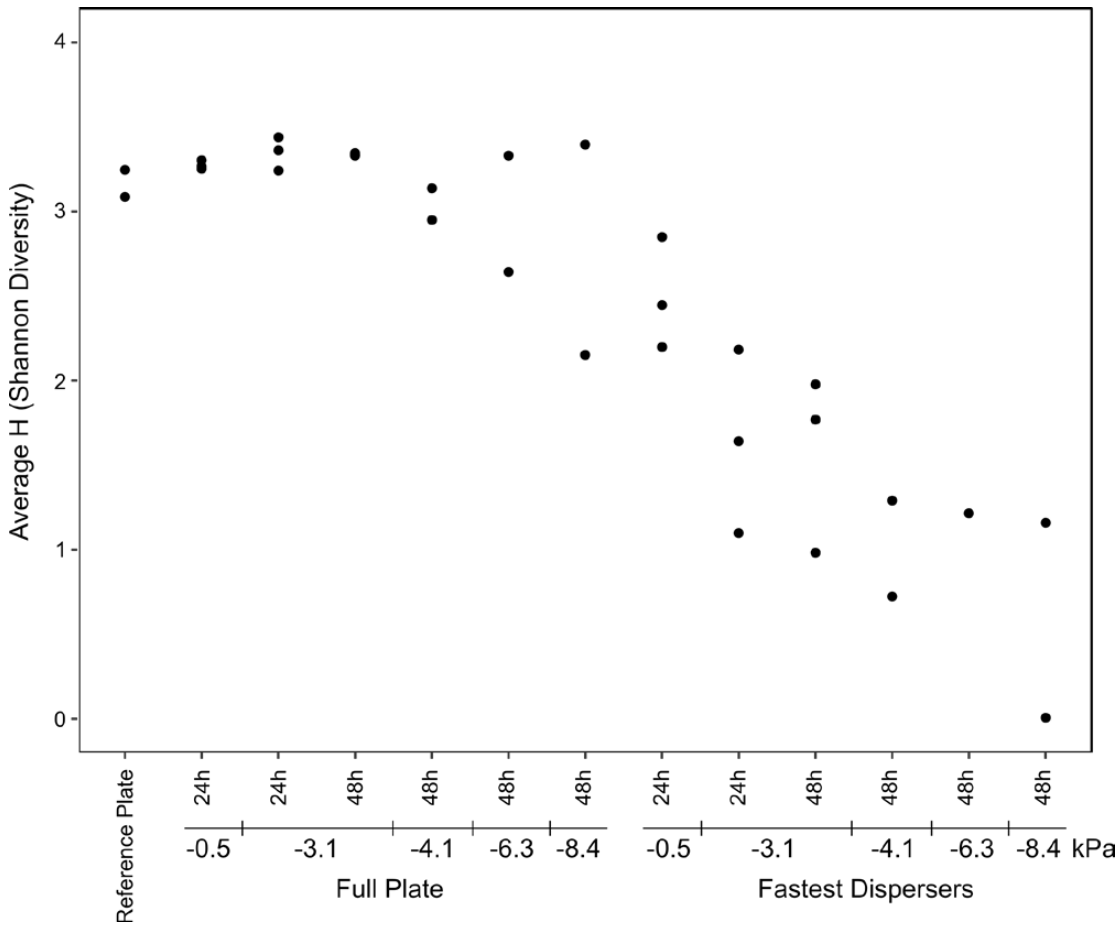
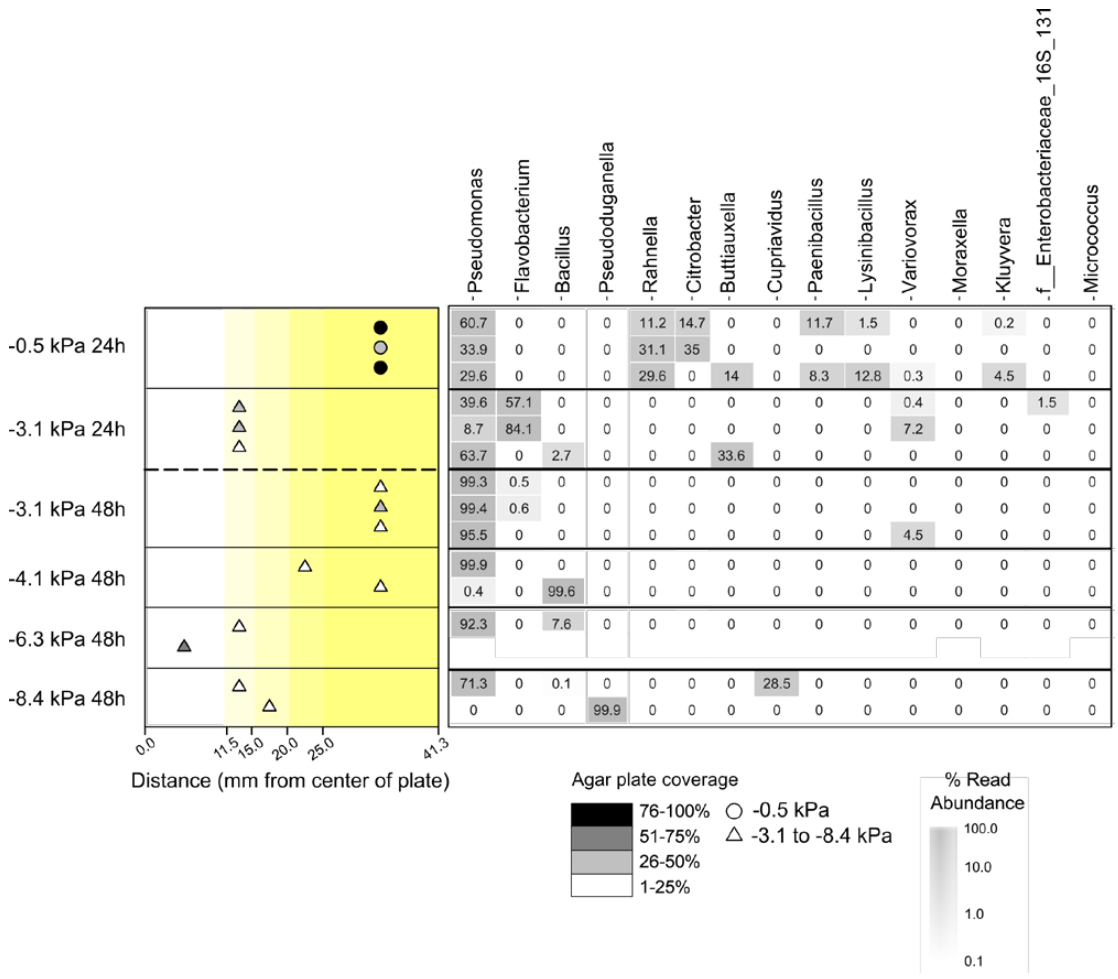


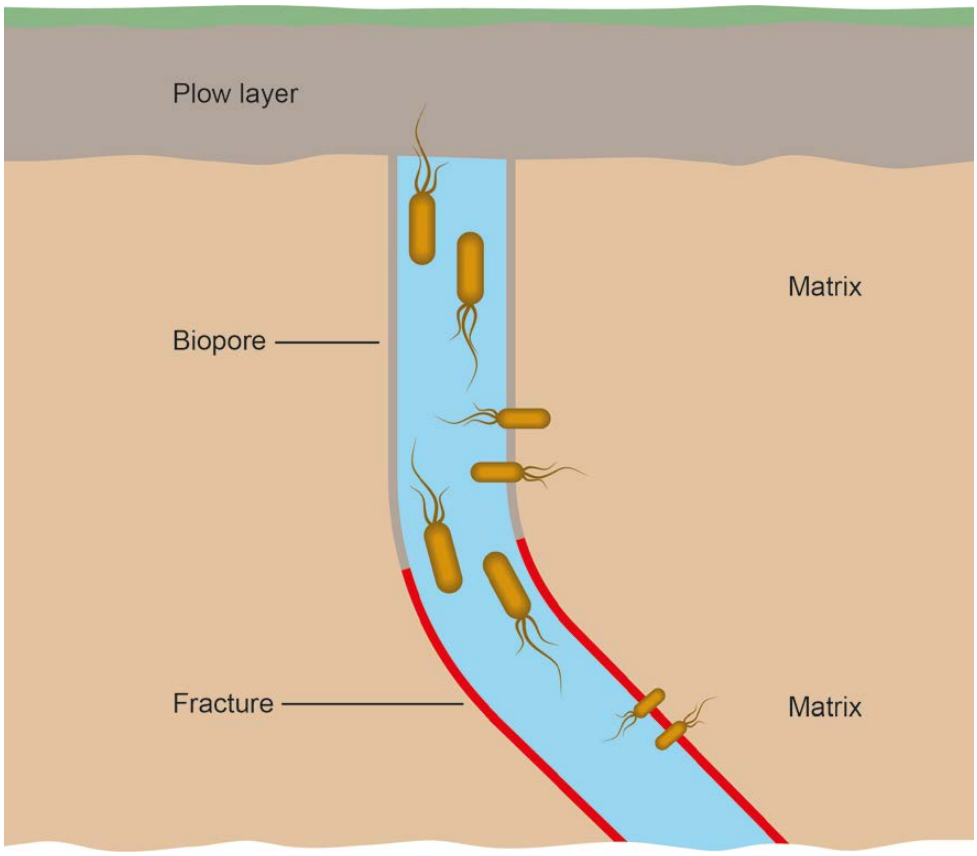
FIG 5 Estimates of alpha-diversity (Shannon diversity index) for communities derived from plow layer soil samples and incubated at a range of negative matric potentials (-0.5 to -8.4 kPa) for 24 h or 48 h. For each replicate PSM, the total community recovered from the full agar plate (full plate) and the dispersed community is presented. A motility-restricted control (reference plate) is also included. Replicates are depicted as separate dots.



734

735 **FIG 6** Dispersal and composition of a community extracted from plow layer soil and incubated at
736 matric potentials from -0.5 kPa to -8.4 kPa for 24 h or 48 h. Left: Symbol shading depicts bacterial
737 coverage of the pressed agar plate, giving an indication of the extent of colonization. The distances
738 shown are ranges, *e.g.* colonies were observed on the agar ring at a distance of between 11.5 to 15
739 mm from the inoculation point at the center. Right: Heatmap of the relative abundance of the
740 most dominant genera among the dispersers. Replication number varied from two to three.

741



742

743 **FIG 7** Conceptual model of preferential flow paths as facilitators of connectivity between
744 communities and "hotspots" for the exchange of motile bacteria. The highest number of shared
745 dispersers were observed along the preferential flow path (plow layer *vs.* biopores and biopores
746 *vs.* fracture), fewer shared dispersers between the biopores and matrix, and almost none shared
747 between the fracture and deep matrix. The size of the depicted bacteria represents the intensity
748 of shared dispersers between compartments.

749

750

751